DESCRIPTION

VACCINE PREPARATIONS CONTAINING ATTENUATED TOXIN

Technical Field

The present invention relates to vaccine preparations that contain an attenuated toxin as the adjuvant and that are useful for treating and preventing diseases.

Background Art

Vaccines have been used to prevent various diseases, and tremendous results have been obtained. Nonetheless, vaccines also have side effects and there are many cases in which vaccines are not very effective. Thus, there is a strong need for improved vaccines.

Currently, many types of vaccines, for human or other animals, use pathogens or parts thereof as antigenic materials for vaccines. Thus, there is no denying the possibility that vaccines may be contaminated with constituents of pathogens or ingredients of the growth medium for the pathogens. These contaminants can provoke undesirable side reactions in vaccination. In addition, antigenic domains themselves, pertaining to immunity, can induce side reactions when inoculated in large quantity.

In an effort to avoid such side reactions as much as possible and manufacture vaccines excellent in safety, those in the art have reduced the inoculum size of vaccine antigen, improved the purity of antigen for vaccination, and/or administered a vaccine by a route other than injection. However, generally such revision results in the reduction of immunogenic activity of the vaccine. Previously, adjuvants have been used as an effective countermeasure to this problem. However, there still remain some problems to be solved, such as improving the effectiveness and safety of the adjuvant.

So far most vaccine inoculations are given by injection. This results in elevation of antibody titer in the blood. When the titer is maintained at a high level, it inhibits the propagation of pathogenic microorganisms and thereby preventing diseases associated with the organisms. On the other hand, various viruses, such as influenza virus,

as well as bacteria infect *via* mucous membranes of the respiratory tract. To prevent such diseases at an early stage of infection, vaccines capable of significantly enhancing local immunity, in the mucous membrane rather than in the blood, are preferred. To achieve this goal, excellent adjuvants that help potentiate local immunity are desired. In other words, the development of an excellent adjuvant, that is effective and safe and that matches the type of antigen and vaccination route to be used, is an important subject in the development of new and improved vaccines.

Noteworthy inoculation routes other than injection include oral, percutaneous and intranasal administration. Injections must be conducted by medical staffs. Therefore, the vaccine inoculation by injection becomes problematic, for example, when it is necessary to vaccinate many people under a condition with poor medical facilities. On the other hand, oral, percutaneous or intranasal administration can be performed with the instruction of a medical professional but without direct assistance as far as vaccine preparations are available. However, in general, sufficient immunological stimulation is hardly attainable with these routes of administration, and therefore suitable adjuvants for such routes are demanded.

Previously, aluminum compounds (aluminum sulfate, aluminum hydroxide, aluminum phosphate, etc.) have been widely used as adjuvants for vaccines. Currently, the gel of aluminum compound is almost the only adjuvant available for vaccines of human use. However, there are some problems in regard to the aluminum adjuvant, and thus improvements are needed. Illustrative problems are pointed out as followings:

1) Problems associated with the production of aluminum adjuvant as well as its handling are as follows: since the quality of aluminum adjuvant tends to vary from one production lot to another, it is not suited to large scale manufacturing. Moreover, the compound tends to be interfering in column chromatography of co-existing proteins, and is therefore inconvenient to handle.

2) Another problem associated with the aluminum adjuvant is that while it excels in potentiating the humoral immunity, the compound is less effective for potentiating cellular immunity. Thus the types of

antigens to be used together are limited.

Research and development of new types of adjuvants, such as bacterial toxins, that overcome the drawbacks discussed above are in progress. Some illustrative examples are as follows:

1. Bacterial toxins, such as cholera toxin, and the like;
2. Substances having detergent activity, such as saponins, higher fatty acids, and the like;
3. Constituents of microorganisms or plants, such as BCG, muramyl peptide, and the like;
4. Functional proteins including cytokines, such as interleukin, heat shock protein, and the like;
5. Synthetic polyanion, polycation, and the like; and
6. Micro-carriers and the like;.

It is well known that certain bacterial toxins have adjuvant activity, namely, activity of enhancing production of antigen-specific antibody. Among them, cholera toxin exhibits relatively high adjuvant activity (J. Holmgren et al., Vaccine 11, 1179-1184, 1993). The present inventors have been studying and developing vaccines containing adjuvants such as cholera toxin, *E. coli* heat-labile toxin, and the subunits thereof (Unexamined Published Japanese Patent Application (JP-A) No. Hei 2-243633).

In addition to these studies, the ongoing research and development, which relates to vaccines containing cholera toxin, *E. coli* heat-labile toxin, pertussis toxin, and such as the adjuvant, includes the following examples: influenza vaccine (K. Komase et al., Vaccine 16, 248-254, 1998; A. S. Tamura et al., Eur. J. Immunol. 21, 1337-1344, 1991), herpes simplex virus vaccine (C. M. Richards et al., Vaccine 15, 1065-1069, 1997), HIV-I vaccine (I. M. Belyakov et al. Proc. Natl. Acad. Sci. USA 95, 1709-1714, 1998), *Helicobacter pylori* vaccine (R. Weltzin et al., Vaccine 15, 370-378, 1997), toxoplasma vaccine (I. Bourgiun et al., FEMS Immunol. Med. Microbiol 12, 121-126, 1995), measles vaccine (C. D. Partidos et al., Imunology 89, 483-487, 1996), poliovirus vaccine, etc.

However, the usage of vaccines added with bacterial toxins as adjuvant in clinical practice might be of problem, because bacterial toxins are originally toxic to humans. The present inventors assumed